

CLAIMS

1. A method of measuring a kinase activity of a cyclin/CDK complex in a sample on RB proteins or partial peptides thereof, comprising the steps of:

(a) contacting a substrate comprising the RB protein with the sample under conditions required for the phosphorylation reaction associated with the kinase to occur; and

(b) detecting changes in the phosphorylation level of the RB protein substrate based on changes in the reactivity of the substrate with antibodies that identify the phosphorylation state of the substrate.

2. A method of screening for compounds that inhibit or enhance the kinase activity of a cyclin/CDK complex on an RB protein, comprising the steps of:

(a) contacting and incubating the cyclin/CDK complex with a substrate comprising the RB protein in the presence of a test compound under conditions required for the phosphorylation reaction to occur;

(b) detecting changes in the phosphorylation level of the RB protein substrate based on changes in the reactivity of the substrate with antibodies that identify the phosphorylation state of the substrate; and

(c) selecting the compound that lowers or elevates the phosphorylation level of the substrate compared to the levels detected in the absence of the test compound.

3. A method of measuring a phosphatase activity on phosphorylated RB proteins in a sample, comprising the steps of:

(a) contacting and incubating a substrate comprising the phosphorylated RB protein with the sample under conditions required for the dephosphorylation reaction to occur; and

(b) detecting changes in the phosphorylation level of the RB protein substrate based on changes in the reactivity of the substrate with antibodies that identify the phosphorylation state of the substrate,

wherein the phosphorylated RB protein is phosphorylated by a

cyclin/CDK complex.

4. A method of screening for compounds that inhibit or enhance the phosphatase activity on phosphorylated RB proteins, comprising the steps of:

- 5 (a) contacting and incubating the phosphatase against the phosphorylated RB protein with a substrate comprising the phosphorylated RB protein in the presence of a test compound under conditions required for the dephosphorylation reaction to occur;
 - 10 (b) detecting changes in the dephosphorylation level of the RB protein substrate based on changes in the reactivity of the substrate with antibodies that identify the phosphorylation states of the substrate; and
 - (c) selecting a compound that lowers or elevates the dephosphorylation level of the substrate compared to the levels detected in the absence of the test compound.
5. The measuring method of any one of Claims 1 to 4, wherein the cyclin is selected from the group consisting of: cyclin A, cyclin B, cyclin D1, cyclin D2, cyclin D3, and cyclin
- 20 E.
6. The measuring method of any one of Claims 1 to 4, wherein the CDK is selected from the group consisting of: CDK 1, CDK 2, CDK 4, and CDK 6.
7. The measuring method of any one of Claims 1 to 4, wherein the cyclin/CDK complex comprises any one of the combinations selected from the group consisting of:
- 25 cyclin A/CDK1;
cyclin A/CDK2;
cyclin B/CDK1;
30 cyclin D1/CDK4;
cyclin D1/CDK6;
cyclin D2/CDK4;
cyclin D2/CDK6;
cyclin D3/CDK4;
35 cyclin D3/CDK6; and
cyclin E/CDK2.

8. The method of Claim 7, wherein the kinase is the cyclin D1/CDK4 complex, and the antibody identifies the phosphorylation state of the serine at position 780 of the RB protein.

9. The method of Claim 1, wherein the individual activities of the cyclin/CDK complexes contained in a sample comprising a plurality of cyclin/CDK complexes that separately phosphorylates different sites can be measured, wherein the antibody that identifies the phosphorylation state identifies the phosphorylation site.

10. The method of any one of Claims 1 to 4, wherein the antibody has a higher reactivity with the phosphorylated RB protein substrate than with a non-phosphorylated RB protein substrate.

11. The method of any one of Claims 1 to 4, wherein the antibody has a higher reactivity with a non-phosphorylated RB protein substrate than with the phosphorylated RB protein substrate.

12. The method of any one of Claims 1 to 4, wherein the substrate is labeled.

13. The method of any one of Claims 1 to 4, wherein substrate is immobilized on a solid phase.

14. The method of any one of Claims 1 to 4, wherein the antibody is labeled.

15. The method of any one of Claims 1 to 4, wherein the phosphorylation level of the substrate is evaluated by the ELISA method.

16. A protein kinase activity regulating agent of the cyclin/CDK complex containing a compound, which can be isolated by the screening method described in Claim 2 as the active ingredient.

17. A protein phosphatase activity regulating agent of a protein phosphorylated by the kinase of a cyclin/CDK complex containing a compound, which can be isolated by the screening method described in Claim 4 as the main principle.

18. The activity regulating agent of Claim 16 or 17, wherein the agent is derived from nature.

19. An immunogen for preparing antibodies used in any of the methods of Claims 1 to 4, containing the RB protein or a peptide comprising an amino acid sequence including one or more phosphorylation sites of the homologues of the RB protein in
5 species other than humans.

20. The immunogen of Claim 19, wherein the phosphorylation site(s) is(are) phosphorylated.

21. The immunogen of Claim 19 or 20, wherein the phosphorylation site is at least one site selected from the
10 group consisting of: threonine at position 356; serine at position 612; serine at position 780; and threonine at position 807 of the human RB protein.

22. The immunogen of Claim 21, wherein the phosphorylation site is selected from the group of amino acid sequences
15 consisting of the sequence shown in SEQ ID NOs: 2, 4, 6, and 8.

23. A kit for performing the methods of Claims 1 to 4, wherein the kit contains an antibody that identifies the phosphorylation state of the substrate.